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Research Article

Seeds and vegetative propagules of all selected invasive plants in Europe do not survive industrial composting

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Abstract

Plant material of invasive alien plant species (IAPS) must be appropriately disposed of to prevent unintended spread. The current guidelines in Slovenia and in several other European countries recommend composting only the parts of the plants from which they cannot sprout and reproduce. At the same time, the vegetative propagules and seeds should be incinerated. We tested whether the seeds and vegetative propagules (rhizomes, stolons, tubers, and branches) of 30 selected IAPS survive industrial composting, which is the method of processing collected organic waste and green cut from parks and gardens. Mature seeds and vegetative propagules were packed in metal boxes, which were filled with compost and included in the hygienisation phase of biowaste processing at the Regional Waste Management Centre, RCERO Ljubljana. After the industrial composting for 17 days, seed germination and viability tests were done and compared with a control group of seeds collected from the same plants but not undergoing the composting process. The composted and fresh vegetative propagules were planted in pots with soil, and the number of rooted parts was counted. None of the seeds and the vegetative propagules survived the industrial composting process, and we can conclude that it is safe to dispose of the IAPS like other organic waste or green cut.

Key words: Brown waste, disposal of plant material, germination, green cut, IAPS waste collecting, invasive alien plants, non-native plants, vegetative reproduction, waste management

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Introduction

Invasive alien plant species (IAPS) are causing environmental and ecological problems and are responsible for substantial biodiversity decline in almost all regions of the planet (Vilà et al. 2011; Bellard et al. 2016; Diagne et al. 2021).

In Europe, preventing and minimising the effects of invasive alien species on biodiversity is one of the programmes of the European Commission, resulting in the Invasive Alien Species Regulation (Regulation (EU) 1143/2014; (European Commission 2014). The regulation contains a List of invasive alien species of Union concern, including 41 plant species in 2024. The listed species are subject to restrictions and measures, including restrictions on keeping, importing, selling, breeding, growing and releasing into the environment. However, not all invasive species present in Europe are regulated. For example, the extremely widespread

species *Reynoutria japonica* Houtt. and North American *Solidago* spp., *Phytolacca americana* L., which have been spreading very effectively in the last decades, and the highly allergenic *Ambrosia artemisiifolia* L., are not (yet) included in the EU list. These and several other invasive or potentially invasive alien species and non-native species from alert lists (Essl and Rabitsch 2002; Celesti-Grapow et al. 2009; De Groot et al. 2017; Csiky et al. 2023; EPPO 2024; Nikolić 2024) are at least for now not regulated by the European legislation. The regulation and management of these IAPS is the domain of each European member state.

The economic costs of biological invasions are incredibly high and are still underestimated (Diagne et al. 2021). The prevention and the response in the early phases of plant invasion are much more effective and less expensive comparing interventions in highly invaded areas (Finnoff et al. 2010). However, the early phases are already over in several regions, and in these cases, we have to face the problem of costly plant removal. Usually, the financial and human resources are limited, and in such cases, recognising the high-impact species and prioritising their management is crucial (Blackburn et al. 2014). Actions to remove invasive plants from nature are already a constant practice worldwide.

There is a lot of research on the most effective removal methods for various plant groups, considering the biology and reproduction strategies of different invasive species. Several such scientific publications can be accessed through the websites of organisations that deal with invasive species, e.g., IUCN (https://iucn.org/our-union/commissions/group/iucn-ssc-invasive-species-specialist-group), CABI (https://www.cabi.org/what-we-do/invasive-species/) or GBIF (https://www.gbif.org), national or regional institutions (ministries, agencies and institutes) and different projects dealing with invasive species, as two LIFE projects from Slovenia, Life OrnamentalIAS: https://zrsvn-varstvonarave.si/blog/projekti/life-ornamentalias/ and Life Artemis: https://www.tujerodne-vrste.info/project-life-artemis/).

Despite the worldwide problems with invasive species and numerous studies on their biology, ecology and management, research on disposal methods is still scarce. We found only a few that were dealing with single species (Hassani et al. 2021; Popovic et al. 2021; Wang et al. 2024) or a small group of ecologically connected species (Meier et al. 2014; Strgulc Krajšek et al. 2020). In publications and webpages, primarily written for the general public or different interest groups (for example, Strgulc Krajšek et al. 2016; Dolenc and Papež Kristanc 2020), instructions are based on practical experiences and not on controlled experiments and commonly include the precautionary principle. Robinson et al. (2017), who analysed the websites about the invasive Japanese knotweed (*Reynoutria japonica*), found conflicting information about the potential socio-economic and ecological problems caused by IAPS and contradictory suggestions about the most appropriate management techniques. Burning the collected material is often suggested if the propagules, such as seeds, tubers, etc., are present. Such an approach is safe but expensive. Still, there was no existing research that would analyse the survival rate of invasive alien plant propagules included in regular industrial biowaste processing. Such research is in the interest of scientists who are frequently asked about the effective and safe methods of IAPS disposal and cannot give complete and reliable answers, and in the interest of the institutions dealing with the biowaste, which strive to simplify the collection and processing procedures.

In Slovenia, biodegradable waste including kitchen and garden waste that residents deposit in designated containers (brown waste) from one-fifth of the

households (ca. 470 000 residents) is collected at Ljubljana Regional Waste Management Centre (RCERO Ljubljana) (Sankovič 2017). They also accept yard waste (green cut) from the maintenance of gardens and parks, such as branches, leaves, grass, and water lilies.

The biowaste processing in RCERO Ljubljana has several phases (Fig. 1) (Sankovič 2017). Biowaste from households and yard waste are separately collected in the reception hall. In the first step, all gathered material is ground. The biowaste from households is then sieved, metal particles are removed and then it is transported to the bioreactor, where the fermentation process takes place. After three weeks in the bioreactor, the material is squeezed and transported to the composting tunnels, where it is mixed with previously ground yard waste. The phase of composting in tunnels is 2 to 3 weeks long, and during it, the temperature should be a minimum of 55 °C for at least 4 consecutive days, or above 65 °C for at least 3 consecutive days to meet the standards for hygienisation from the Decree on the treatment of biodegradable waste and the use of compost or digestate (Ministrstvo za kmetijstvo in okolje 2013), which is in accordance to Directive 2008/98/EC of the European Parliament and of the Council (https://eur-lex.europa.eu/eli/dir/2008/98/oj). After this phase, the biomass is transported to the maturation hall for four weeks, where it is turned three times a week. During the maturation, the material partially dries so it can be sifted and cleared of plastic particles and other impurities, like stones and bones. The produced compost is supplied to companies that mix the substrates for flowers and gardens. A portion of the compost is used within the municipality for park maintenance. Residents and companies that work on landscaping around houses, green roofs, and similar projects also purchase a smaller portion.

There are several other biowaste processors in Slovenia. One of them processes the biodegradable kitchen waste using anaerobic digestion (biogas plant) only, and the fermentation takes place at lower dry matter content. Aerobic phases do not follow this phase. The digestate is treated as wastewater, and compost is not produced. Other processors have composting facilities and an aerobic process for treating biodegradable waste without an anaerobic component.

The practice of separate collection of plant material of selected IAPS was established in Ljubljana in 2018 as part of the Applause project, in the frame of which researchers studied the possibilities of using IAPS as a raw material for various products (Berden 2019). The selected plant species used in a project were collected separately; additionally, a collection bin for mixed IAPS material was available. All unused material from the containers was sent to incineration to prevent the potential spreading of IAPS. Such practice is safe but expensive.

The main goal of our study was to test the safety of disposing seeds and vegetative propagules of selected invasive plant species that are common across Europe in industrial composting at Ljubljana Regional Waste Management Centre (RCERO Ljubljana). We included the seeds and vegetative propagules in one phase of industrial composting, namely composting with active air ventilation, which took place in the composting tunnels. After the exposure we tested if the propagules survived. Based on the results obtained, we wrote new recommendations for the disposal of biomass of invasive alien plant species (IAPS).

To our knowledge, this is the first experiment where seeds and vegetative propagules of so many invasive species were included in the industrial composting to inspect their viability after the process.

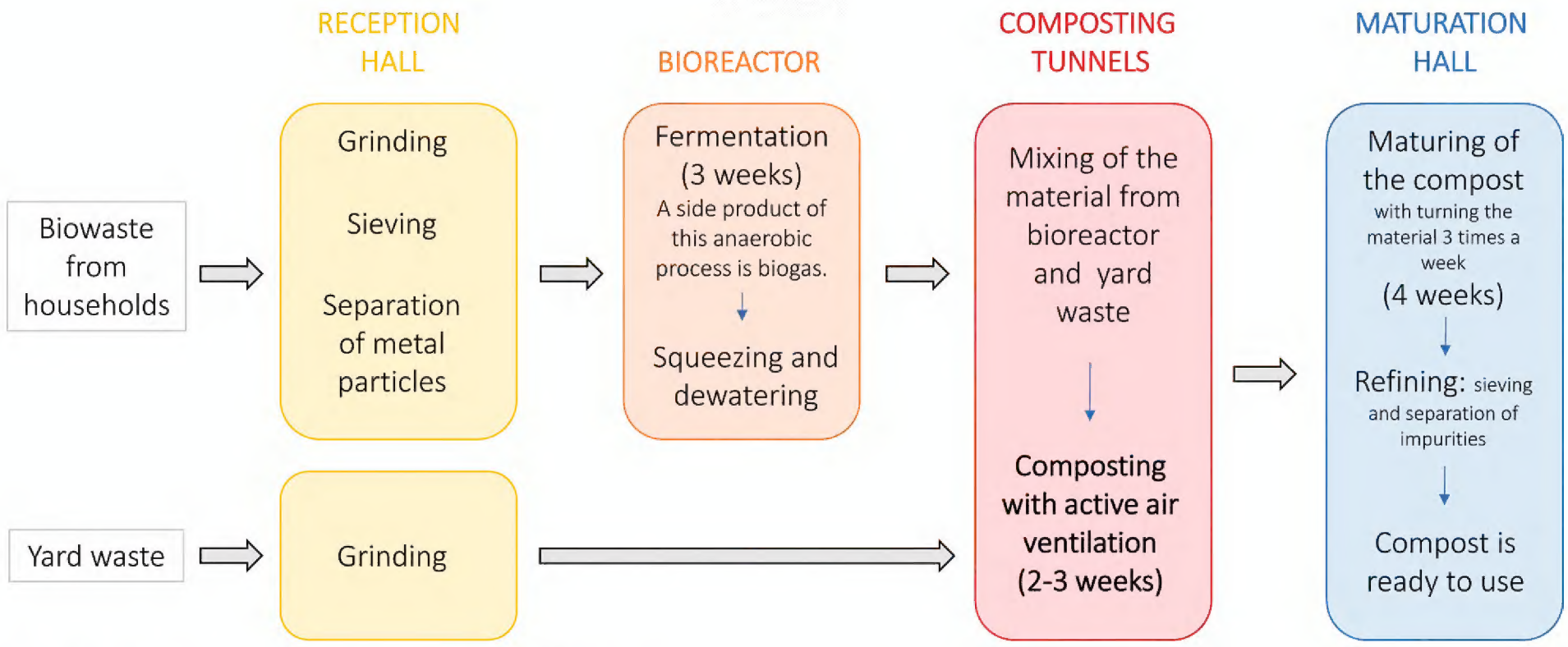
RECEPTION HALL
BIOREACTOR
COMPOSTING TUNNELS
MATURATION HALL
Biowaste from households
Grinding
Sieving
Separation of metal particles
Fermentation (3 weeks)
A side product of this anaerobic process is biogas.
Squeezing and dewatering
Mixing of the material from bioreactor and yard waste
Yard waste
Grinding
Composting with active air ventilation (2-3 weeks)
Maturing of the compost with turning the material 3 times a week (4 weeks)
Refining: sieving and separation of impurities
Compost is ready to use

Figure 1. The biowaste processing in RCERO Ljubljana.

Materials and methods

Plant material

The propagules of 30 IAPS, present in Slovenia were tested, 26 as seeds or fruits and 11 as vegetative propagules (Table 1). We included trees and shrubs, vines, perennials and annuals, so we covered a variety of life forms.

The material for the experiment with seeds

We selected 26 invasive alien species already widespread in Europe (Table 1). In September and October 2022, we collected their ripe seeds or fruits, depending on the species' propagule. All material was collected in Slovenia. Additionally, we used the seeds of two commercially available species, *Lactuca sativa* L. and *Raphanus sativus* L. (Royal Seeds, HortuSì srl, Italy, https://hortus.org), as a control. Seeds and fruits were dried at room temperature and stored in a dry and dark place at room temperature until they were used.

Before the experiment, we divided plant seeds or fruits of all species into three batches: C–control, T1–first composting, and T2–second composting. The division of propagules into batches was random; however, each batch contained approximately the same number of fruits or seeds. Propagules of control batches (C) were stored in covered but not sealed Petri dishes and placed in a dry and dark place at room temperature. The propagules of test groups T1 and T2 were packed in parcels made of nylon socks. The unique combination of small plastic cubes with letters served as a code for identifying the samples after the composting.

The material for the experiment with vegetative propagules

In March 2024, we collected the vegetative parts that serve as propagules for vegetative reproduction of 11 IAPS (Table 1). We cut the one or two-year-old branches into ca. 15 cm long pieces and washed the underground parts. We divided all the material into three batches: C–control, T1–first composting, and T2–second composting. Each batch contained approximately the same amount of the plant

Table 1. Invasive plant species included in the experiments. Legend to the assigned invasiveness status: EU - the species is on the List of invasive alien species of Union concern (European Commission 2014), EPPO - the species is declared invasive in Europe (EPPO 2024), ISSG - the species is included in the Global Register of Introduced and Invasive Species for at least one European country (GBIF 2024), SLO - the species is on the Slovenian list of IAPS (Strgulc Krajšek et al. 2016).

Species	Invasiveness status	Locality (country, town, coordinates)	Date of collection	Composted part
Acer negundo L.	ISSG, SLO	Slovenia, Ljubljana, 46.048724, 14.603788	1. 10. 2022	Fruits
		Slovenia, Ljubljana, 46.065500, 14.507917	15. 3. 2024	Branches
Ailanthus altissima (Mill.) Swingle	EU, EPPO, ISSG, SLO	Slovenia, Kranj, 46.244287, 14.356486	14. 9. 2022	Fruits
Ambrosia artemisiifolia L.	EPPO, ISSG, SLO	Slovenia, Vikrče, 46.126819, 14.445264	1. 10. 2022	Fruits
Amorpha fruticosa L.	EPPO, ISSG, SLO	Slovenia, Ljubljana, 46.041521, 14.514444	22. 9. 2022	Fruits
Asclepias syriaca L.	EU, EPPO, ISSG, SLO	Slovenia, Ljubljana, 46.070490, 14.524841	1. 10. 2022	Seeds
Berberis thunbergii DC.	ISSG, SLO	Slovenia, Kranj, 46.243899, 14.356559	14. 9. 2022	Fruits
Buddleja davidii Franch.	EPPO, ISSG, SLO	Slovenia, Kranj, 46.245129, 14.357795	14. 9. 2022	Fruits
		Slovenia, Ljubljana, 46.065528, 14.472444	14. 3. 2024	Branches
Cornus sericea L.	EPPO, ISSG, SLO	Slovenia, Ljubljana, 46.053210, 14.470126	20. 9. 2022	Fruits
			14. 3. 2024	Branches
Echinocystis lobata (Michx.) Torr. & A.Gray	ISSG, SLO	Slovenia, Šmartno, 46.053063, 14.470288	2. 10. 2022	Seeds
Erigeron annuus (L.) Pers.	ISSG, SLO	Slovenia, Rakovica, 46.250422, 14.322822	29. 9. 2022	Fruits
Helianthus tuberosus L.	EPPO, ISSG, SLO	Slovenia, Ljubljana, 46.117162, 14.449836	3. 9. 2022	Tubers
Impatiens glandulifera Royle	EU, EPPO, ISSG, SLO	Slovenia, Ljubljana, 46.052692, 14.470819	20. 9. 2022	Seeds
Lonicera maackii (Rupr.) Maxim.	ISSG, SLO	Slovenia, Ljubljana, 46.065806, 14.470667	14. 3. 2024	Branches
Mahonia aquifolium (Pursh) Nutt.	ISSG, SLO	Slovenia, Ljubljana, 46.038189, 14.513496	20. 9. 2022	Fruits
Parthenocissus quinquefolia (L.) Planch.	ISSG, SLO	Slovenia, Kokrica, 46.262577, 14.356993	20. 9. 2022	Fruits
		Slovenia, Ljubljana, 46.061917, 14.468472	14. 3. 2024	Branches
Paulownia tomentosa (Thunb.) Steud.	ISSG, SLO	Slovenia, Ljubljana, 46.262577, 14.356993	2. 10. 2022	Seeds
Physocarpus opulifolius (L.) Maxim.	ISSG, SLO	Slovenia, Kokrica, 46.262171, 14.353872	14. 9. 2022	Seeds
Phytolacca americana L.	ISSG, SLO	Slovenia, Medno, 46.052692, 14.470819	3. 9. 2022	Fruits
Potentilla indica (Andrews) Th.Wolf	ISSG, SLO	Slovenia, Ljubljana, 46.053063, 14.470288	20. 9. 2022	Aggregate fruits
Prunus laurocerasus L.	ISSG, SLO	Slovenia, Ljubljana, 46.040230, 14.512205	20. 9. 2022	Fruits
Reynoutria japonica Houtt.	EPPO, ISSG, SLO	Slovenia, Ljubljana, 46.036528, 14.528306	1. 10. 2022	Fruits
		Slovenia, Stanežiče, 46.110611, 14.445639	14. 3. 2024	Rhizomes
Reynoutria × *bohemica* Chrtek & Chrtková	EPPO, ISSG, SLO	Slovenia, Ljubljana, 46.117162, 14.449836	14. 3. 2024	Rhizomes
Rhus typhina L.	ISSG, SLO	Slovenia, Kranj, 46.249424, 14.355008	14. 9. 2022	Fruits
Robinia pseudoacacia L.	ISSG, SLO	Slovenia, Kranj, 46.241528, 14.355463	14. 9. 2022	Seeds
Rosa multiflora Thunb.	ISSG, SLO	Slovenia, Ljubljana, 46.042274, 14.515662	20. 9. 2022	Aggregate fruits
Rudbeckia laciniata L.	ISSG, SLO	Slovenia, Ljubljana, 46.061917, 14.468889	14. 3. 2024	Rhizomes
Solidago canadensis L.	EPPO, ISSG, SLO	Slovenia, Ljubljana, 46.117162, 14.449836	2. 10. 2022	Fruits
		Slovenia, Ljubljana, 46.065667, 14.471667	14. 3. 2024	Rhizomes and stolons
Solidago gigantea Aiton	EPPO, ISSG, SLO	Slovenia, Ljubljana, 46.052692, 14.470819	20. 9. 2022	Fruits
		Slovenia, Ljubljana, 46.117162, 14.449836	14. 3. 2024	Rhizomes and stolons
Spiraea japonica L.fil.	ISSG, SLO	Slovenia, Šmarna gora, 46.122526, 14.456736	1. 10. 2022	Seeds
Symphoricarpos albus (L.) K.Koch	ISSG, SLO	Slovenia, Kokrica, 46.262296, 14.356340	17. 10. 2022	Fruits

material. We wrapped the plant material from the test groups (T1 and T2) into garden cover fabric (Vrteks, Tosama) together with a unique combination of small plastic cubes with letters that served as a code and stored the parcels in plastic bags in the fridge at 4 °C until use.

Composting procedure

Two boxes with walls made of perforated steel plates were constructed for the composting experiment. The diameter of the round holes in steel plates was 4 mm. The interior of the box was lined with thin wire mesh with a hole size of 1.5 mm. A metal chain was fixed on the side of each box to mark the box's position in the compost pile. All parcels with plant material from one batch (T1 or T2) were mixed with dry compost and placed into the box. The box was covered with a perforated cover made of the same material as the box.

Boxes with parcels from all batches were included in industrial composting. In the years 2022–2023, we composted seeds, and in 2024 the vegetative propagules. The two boxes with seeds (seed batches T1 and T2) were composted in two different compost tunnels, and the boxes with vegetative propagules (vegetative propagules batches T1 and T2) were in the same compost pile but buried at different positions, as we wanted to avoid the risk of losing some of the material due to drying or decomposition in the time of waiting for the next composting pile to be ready for the start of the composting procedure. All the composting procedures were 17 days long.

The temperature regimes during the composting of seeds (batches T1 and T2) were measured with temperature probes, positioned near each of the boxes. The temperature probe for measuring the temperature regime during the composting of vegetative propagules was positioned between the boxes. In all cases, the temperature was recorded once per hour. The composting procedures in all three compost piles were similar, with some differences in temperature regimes (Fig. 2), but all were in accordance with the valid legislation (Ministrstvo za kmetijstvo in okolje 2013).

The composting piles were ventilated through ground-positioned air nozzles in two ways: with air pressure and suction to regulate the temperature in compost piles. For detailed information, see Suppl. materials 1, 2.

Germination and seed viability tests

After composting, the seeds were spread to open Petri dishes and dried at room temperature for a few days. By drying the seeds, we imitated the compost maturation phase in the maturation hall, during which the compost matures and dries (Fig. 1). Afterwards, we covered the Petri dishes and placed all the seeds, including the control batch, which was stored at room temperature until then, in the refrigerator at 4 °C for 2 months to break the potential dormancy of seeds.

Before the viability tests, we isolated seeds from fruits or their decomposed parts. All seeds were also washed with running tap water. Some seeds from the series T1 and T2 have partly decomposed or damaged during the composting. In those cases, visually, the complete seeds were selected for germination experiments. We scarified the seeds of *Robinia pseudoacacia* with a scalpel and crushed the *Prunus laurocerasus* pit before including them in the germination experiment.

The germination of most of the seeds was tested in Petri dishes on filter paper watered with tap water. The petri dishes were kept in growing chambers at 24 °C, 12 hours of light per day and 50% air humidity. The *Ambrosia artemisiifolia* seeds

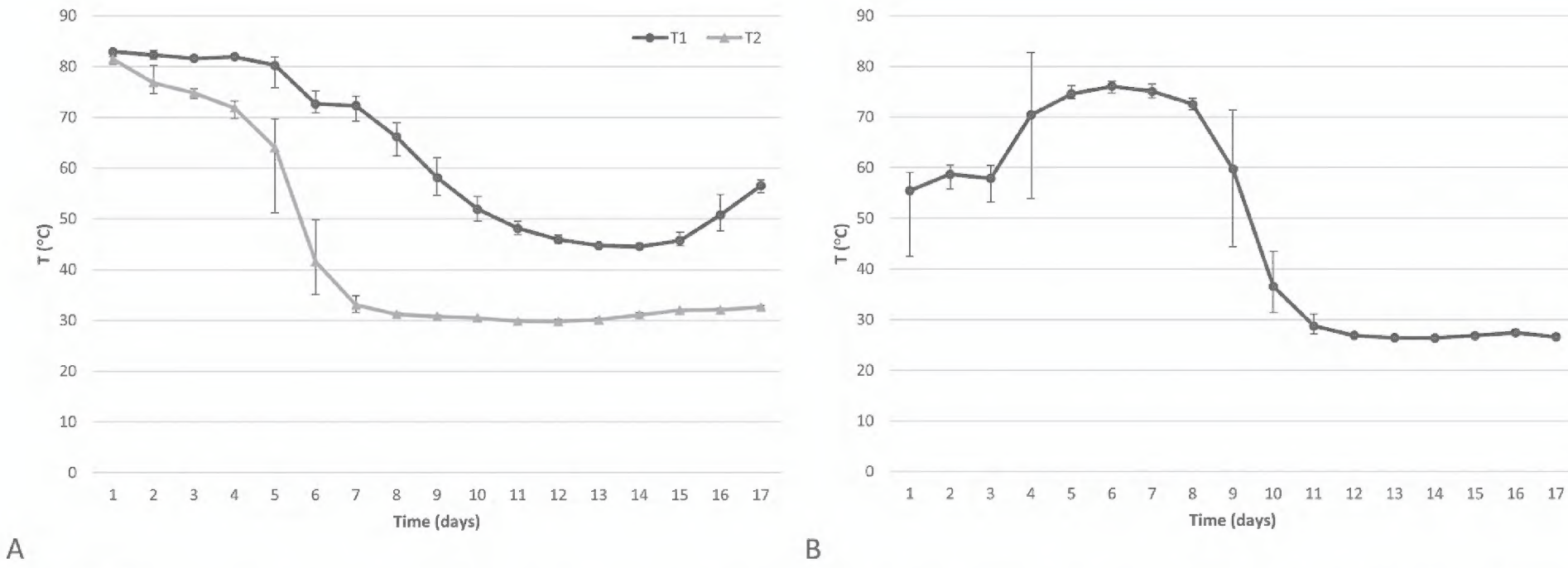

Figure 2. Average day temperature in the compost pile beside the boxes with seeds of batch T1 (black line) and T2 (grey line) (**A**) and during the composting of the vegetative propagules (**B**). Vertical bars show minimum and maximum temperatures.

were moved from the growing chamber to the fridge (4 °C) every night. In some cases, we used germination trays filled with soil. The planters were covered with transparent plastic covers and kept in the same conditions as Petri dishes. The trays were watered with tap water twice a week when we observed that the soil started to dry. This method was also used when the germination tests in Petri dishes were unsuccessful. The information about the length and the types of germination experiments is presented in Tables 2, 3.

In cases when control seeds did not germinate, we tested the potential viability of the seeds using the Tetrazolium viability test (Table 3) (Cottrell 1947). We randomly selected the subsample of seeds that did not germinate, cut the seeds in half, placed cut seeds in the holes of microplates, then covered them with a few drops of 1% triphenyl tetrazolium chloride (TTC) and kept them in the dark at room temperature for 20 h. The embryos that were coloured dark red after the incubation were counted as viable.

Testing the viability of propagules for vegetative reproduction

The vegetative propagules were planted in marked box planters filled with commercially available universal non-acidic soil for plants (Substral, Slovenia).

The vegetative propagules of the control group were planted the same day as the material was collected. Rhizomes and tubers were put in the container on the soil and covered with an approximately 2 cm thick layer of soil. We stuck the ca. 5 cm long lower part of the cut branches into the soil. The upper parts stayed above ground. The box planters were left uncovered in an indoor place with natural light at 22 °C in Ljubljana for 2 months (middle of March to middle of May). The light regime was natural, twice a week watering with tap water to prevent the soil from drying out.

The composted material was planted in the same way as the control, but one month later, so the experiment took place from the middle of April to the middle of June. The conditions were the same as for the control, but the light period was longer because the box planters were in the room with natural light. Each box planter contained one species's material and from one treatment only. The quantities of the planted vegetative propagules are given in Table 4.

Table 2. Germination of seeds of invasive plant species and two commercially available crop species before and after composting. Control seeds are seeds that were not composted, T1 and T2 represent two batches of composted seeds.

Species	Germination test (S-soil, P-Petri dish)	Duration of the experiment [days]	Number of seeds per test (replicates)	Germination [%]		
				Control	T1	T2
Acer negundo	S	14	25 (5)	68	0	0
Ailanthus altissima	S	57	25 (5)	0	0	0
Ambrosia artemisiifolia	P	21	100 (4)	34	0	0
Amorpha fruticosa	P	14	25 (5)	44	0	0
Asclepias syriaca	P	28	100 (4)	0	0	0
Berberis thunbergii	P	14	100 (4)	0	0	0
	S	42	100 (4)	0	0	0
*Buddleja davidii**	S	21	not counted (4)	22*	0	0
Cornus sericea	P	14	100 (4)	0	0	0
	S	42	100 (4)	0	0	0
Echinocystis lobata	P	35	10(2)	0	0	0
Erigeron annuus	P	7	100 (4)	90	0	0
Impatiens glandulifera	P	14	50 (2)	6	0	0
Mahonia aquifolium	P	28	100 (4)	0	0	0
Parthenocissus quinquefolia	P	28	100 (4)	0	0	0
Prunus laurocerasus	P	56	100 (4)	0	0	0
Paulownia tomentosa	P	12	100 (4)	87	0	0
Physocarpus opulifolius	P	21	100 (4)	17	0	0
Phytolacca americana	P	56	50 (2)	46	0	0
Potentilla indica	P	28	100 (4)	36	0	0
Reynoutria japonica	P	21	100 (4)	0	0	0
Rhus typhina	P	35	100 (4)	0	0	0
Robinia pseudoacacia	P	7	25 (5)	88	0	0
Rosa multiflora	P	35	100 (4)	0	0	0
Solidago canadensis	P	15	100 (4)	21	0	0
Solidago gigantea	P	20	100 (4)	48	0	0
Spiraea japonica	P	12	100 (4)	97	0	0
Symphoricarpos albus	P	14	100 (4)	0	0	0
	S	42	100 (4)	0	0	0
Lactuca sativa	P	6	50 (2)	96	0	0
Raphanus sativus	P	6	50 (2)	84	0	0

*In the case of *Buddleja davidii*, the number of seeds was not counted because they were not recognisable after the composting. Therefore, the unknown number of seeds was sown, and the number marked with a star symbol represents the number of seedlings, not the percentage of germinated seeds.

Table 3. Metabolic activity of seeds of selected invasive plant species before and after composting, obtained from the Tetrazolium test. Control seeds are seeds that were not composted, T1 and T2 represent two batches of composted seeds.

Species	Number of tested seeds	Viable seeds [%]		
		Control	T1	T2
Ailanthus altissima	24	25	0	0
Asclepias syriaca	24	75	0	0
Berberis thunbergii	24	54	0	0
Cornus sericea	24	63	0	0
Echinocystis lobata	10	100	0	0
Reynoutria japonica	20	0	0	0
Mahonia aquifolium	24	92	0	0
Parthenocissus quinquefolia	24	100	0	0
Prunus laurocerasus	24	21	0	0
Rhus typhina	24	0	0	0
Rosa multiflora	24	25	0	0
Symphoricarpos albus	24	8	0	0

Results

Germination and seed viability

The collected seeds of all species except *Reynoutria japonica* and *Rhus typhina* were viable according to the germination experiments or the Tetrazolium viability tests. However, none of the seeds from series T1 and T2 survived the composting. The results of the tests are shown in Tables 2, 3.

The viability of propagules for vegetative reproduction

All the vegetative propagules of the selected invasive plant species, except the branches of *Cornus sericea*, were viable. At least some of the vegetative propagules used in the control experiment developed roots and green shoots (Table 4). The industrial composting destroyed all the viable parts of the plant material and none of the planted composted fragments developed roots or shoots (Table 4).

Table 4. Vegetative propagation of selected invasive plant species before and after industrial composting. Control are the propagules that were not composted, T1 and T2 represent two batches of composted vegetative propagules. Legend: T-tuber, R-rhizome, B-branch.

Species	Propagule	Number of propagules	Number of viable propagules with roots (total number of shoots)		
			Control	T1	T2
Acer negundo	B	8	3(3)	0	0
Buddleja davidii	B	18	4(6)	0	0
Cornus sericea	B	14	0(0)	0	0
Helianthus tuberosus	T	9	9(15)	0	0
Lonicera maackii	B	7	6(6)	0	0
Parthenocissus quinquefolia	B	14	6(6)	0	0
Reynoutria × bohemica	R	7	5(15)	0	0
Reynoutria japonica	R	5	5(7)	0	0
Rudbeckia laciniata	R	6	6(22)	0	0
Solidago canadensis	R	7	7(22)	0	0
Solidago gigantea	R	10	10(15)	0	0

Discussion

Of the seeds or vegetative propagules of 30 different plant species that are invasive in Slovenia and Europe, none survived the industrial composting in composting tunnels at RCERO Ljubljana. The propagules of IAPS may occur in two sources of biowaste commonly collected in Ljubljana: brown waste, which is collected in households, and yard waste from the maintenance of gardens and parks. Households usually dispose of smaller amounts of plant biomass removed from gardens and put them in bins for organic waste. However, the material from parks and public areas and the collected material of IAPS during public actions are usually transported directly to the collection centres as the green cut.

The phase of composting in tunnels is common to both sources of biowaste (Fig. 1), and this is the reason that we decided to include the propagules in this phase only. In this phase, the temperatures rise above 70 °C for a few days (Fig. 2). For optimal composting long periods of high temperatures must be avoided to allow the development of eumycetes and actinomycetes, which are the main decomposers of the long-chain polymers, cellulose and lignin (de Bertoldi et al. 1983). The most effective way to control the temperature is forced pressure ventilation and turning the composting material (de Bertoldi et al. 1983).

The biowaste from households in RCERO Ljubljana also goes through the anaerobic fermentation phase, where the temperature is 55(±1)°C, and this phase can additionally reduce the chance of survival of seeds and vegetative propagules. However, in the commercial mesophile bioreactors, the temperatures are lower. In the experiment made by Hahn et al. (2023), where T did not exceed 42 °C, hard-seed species, such as *Malva sylvestris* L., survived the procedure, but the seeds with softer testa, such as *Chenopodium album* L., were completely inactivated by the treatments. Johansen et al. (2013) exposed seeds to anaerobic digestion at higher temperatures (55 °C) and complete mortality of all 7 tested plant weeds after two days. They report that the temperature seemed to be the major cause of damage, as the same species germinated after the anaerobic digestion at 37 °C. The survival of seeds after the anaerobic digestion at 37 °C was reported also by Westerman et al. (2012).

The reason that seeds and vegetative propagules did not survive the process of hygienisation must be the combination of high temperatures, humid environment, high concentration of ammonia, pathogen infestations, water-soluble organic phytotoxins, and microorganisms in the substrate, as was already reported by Hassani et al. (2021), who did the composting experiment of *Lupinus polyphyllus* Lindl. seeds, and several others, for example, Zaller (2007), Eghball and Lesoing (2000) and Johansen et al. (2013). High temperature is most probably not enough to kill the seeds of all species. Seeds of different plant species have different tolerance to high temperatures. So, several of the tested IAPS may have seeds sensitive to temperatures higher than 55 °C. The extreme tolerance was reported by Daws et al. (2007), who discovered that seeds of some desert succulents survive exposure to 103 °C for 17 hours. The seeds of the black locust (*Robinia pseudoacacia*), which we tested in our experiment, can survive the increased temperatures during the fire. Popovic et al. (2021) exposed the seeds of black locust to 100 °C for 2.5 and 5 minutes and tested their germination. It was significantly lower compared to the control seeds, but several seeds survived the exposure. In our experiment, black locust seeds did not survive the composting process despite lower temperatures during the process (Fig. 2). Even the lower temperature, at least 57.2 °C, was enough to destroy the propagules of aquatic invasive plant species *Arundo donax* L., *Hydrilla verticillata* (L.f.) Royle, *Eichhornia crassipes* (Mart.) Solms, and *Pistia stratiotes* L. from the Rio Grande River, during the large-scale composting experiment (Meier et al. 2014). In this experiment, the whole plants containing vegetative propagules and seeds were composed, and the result was a valuable compost product without the viable propagules of IAPS.

In our experiment, the seed viability of the control group and both batches of composted seeds was tested by the combination of a germination test and a test of metabolic activity by Tetrazolium staining, which was used when the control seeds did not germinate (most probably because of dormancy or unsuitable germination conditions). None of the methods have shown that the seeds would survive composting. The control seeds of all species, except *Rhus typhina* and *Reynoutria japonica*, were viable. We found that all the seeds of *Rhus typhina* were empty. We checked the seeds from another locality, and they were empty, too. In Slovenia, we have not yet observed the propagation of this species by seeds, but vegetative propagation with stolons is very common. The ripe fruits of *Reynoutria japonica* were collected at the site, where we had already collected the viable seeds for other experiments. Hence, the result that the seeds were not viable was unexpected. Regarding the results of other tested IAPS, we do not expect that viable *Reynoutria*

japonica seeds will survive composting. Similarly, the branches of *Cornus sericea* of the control group did not grow roots. *Cornus sericea* is a species that vegetatively reproduces mainly by ground layering, where the stem is bent down and partly buried in the soil while still attached to the parent plant (Bačič et al. 2015). However, cut branches also can root and serve as vegetative propagules (Strgulc Krajšek et al. 2020). Any other branches or rhizomes that we used in the experiment did not survive the composting. The vital parts of all branches, tubers and rhizomes were almost completely decomposed, only the wooden parts and bark remained.

Based on the fact that 100% of the tested species were inactivated during composting, we believe that we can state with high probability that the industrial composting of IAPS is safe in terms of preventing the spread of IAPS into nature by using the obtained compost, when the composting process meets the requirements of "Decree on the treatment of biodegradable waste and the use of compost or digestate" (Ministrstvo za kmetijstvo in okolje 2013) and Directive 2008/98/EC of the European Parliament and of the Council (https://eur-lex.europa.eu/eli/dir/2008/98/oj).

New recommendations for the disposal of IAPS biomass can be summarised in the following two points:

1. Plant material without seeds or vegetative propagules can be composted in garden compost heaps or disposed of as other household organic waste or green waste from parks and gardens. Disposing of any garden plant material in natural environments, such as riverbanks, forests, or forest edges, must be prohibited.
2. Plant material containing seeds (even if not fully ripe) or vegetative propagules (such as rhizomes, bulbs, tubers, and branches that can regrow) should be disposed of as household organic waste (brown waste) or yard waste from parks and gardens intended for industrial composting. When composting process complies with the standards set by the "Decree on the treatment of biodegradable waste and the use of compost or digestate" (Ministrstvo za kmetijstvo in okolje 2013) and Directive 2008/98/EC of the European Parliament and of the Council (https://eur-lex.europa.eu/eli/dir/2008/98/oj), there is no risk of spreading IAPS through the use of the produced compost.

Conclusion

We used the seeds or vegetative propagules of 30 different IAPS that are invasive in Slovenia. We included trees and shrubs, vines, perennials and annuals, so we covered a variety of life forms. As there were no observed viable propagules after the composting, we proposed the new management recommendation that waste from IAPS can be composted and does not have to be incinerated. This less complicated protocol may simplify and reduce the costs of IAPS disposal and can have another positive effect, namely that more residents will choose to dispose of plant material of IAPS in bins for organic waste or bring it to landfills, and less of the IAPS will end up in compost heaps in the wild, that is still a common practice in Slovenia (Šipek and Šajna 2020).

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

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Author contributions

Conceptualization: SSK. Formal analysis: NG, ST. Funding acquisition: SSK. Investigation: NG, SSK, ST, MR. Methodology: MR, SSK, ST. Resources: SSK. Supervision: SSK. Validation: SSK. Visualization: SSK. Writing - original draft: ST. Writing - review and editing: SSK, NG, MR.

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Data availability

All of the data that support the findings of this study are available in the main text or Supplementary Information.

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Supplementary material 1

The information about the composting procedures

Authors: Marjeta Resnik

Data type: xlsx

Explanation note: The file consists of three spreadsheets, each containing one table with the parameters measured during the composting procedures. The parameters are: retention time, temperature, duration of ventilation with air pressure, and duration of ventilation with air sucction.

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Link: https://doi.org/10.3897/neobiota.97.133943.suppl1

Supplementary material 2

Daily duration of the two types of ventilation during composting of seeds (TS-1 and TS-2) and vegetative propagules (T-V)

Authors: Marjeta Resnik

Data type: docx

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